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Uses and limitations of monoclonal antibodies (MoAbs) in the treatment of malignant disease: a review

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Introduction

In the late 1970s and early 1980s, monoclonal antibodies (MoAbs) were considered to have the potential of revolutionizing cancer therapy. However, the theoretical advantage these reagents have in enhancing the therapeutic index (relative uptake in tumour as compared to normal tissues) has not materialized in practice. Three major limitations of murine MoAbs have been observed in clinical studies; the lack of uptake in solid tumour deposits, the immunogenicity of the reagents causing problems with repeated administration, and non-specific uptake of MoAbs into normal organs. These problems are independent of the targeted agent and thus relate to the antibodies themselves. As a consequence, MoAbs have been developed in other species such as rat and hamster, and murine MoAbs have been 'humanized' to change the pharmacokinetics and biodistribution of the reagents. In addition, MoAbs have been modified to make them smaller, with proteolytic digestion yielding $(Fab)_2$ and Fab fragments and molecular techniques allowing the synthesis of fragments representative of the amino acids forming the antigen binding site within the immunoglobulin molecule (CDRs)[1]. Here we review approaches to targeting cytotoxic agents to cells, the limitations that have been encountered and current therapeutic strategies developed to overcome these problems.

Cytotoxic materials conjugated to MoAbs

Radioisotopes, cytotoxic drugs and toxins represent the major groups of materials conjugated to MoAbs for therapeutic studies, although other compounds such as nucleic acids, complex carbohydrates and photoactivators have also been used. Most clinical experience has been gleaned with radionuclides conjugated to MoAbs, because it is often possible to detect simply the biodistribution of the material within an individual by gamma camera scintigraphy. Considerable data also now exists on the use of immunotoxins administered systemically.

Isotopes of iodine conjugated to MoAbs have predominantly been used for either the diagnosis or treatment of maligancies. ^{123}I has been used solely for scintigraphic studies, whereas ^{131}I is used for both diagnosis and therapy. The advantages that iodine offers over other isotopes is the simple chemistry needed to link it to the MoAb. However, for therapy the emissions from ^{131}I are far from ideal (see below). Other isotopes employed clinically include ^{111}Indium and ^{99}Technetium for imaging studies and ^{90}Yttrium for immunotherapy. The chemistry of linking these radionuclides to MoAbs is much more complex than for ^{131}I and the stability of the conjugates in vivo can also be problematical. Developments to enhance the stability of the metal chelates in vivo include the generation of macrocycles; compounds designed to

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hold the isotopes in a 'cage' attached to the MoAb. Unfortunately, the linkers used to attach the macrocycles to MoAbs often prove highly immunogenic in patients and this currently limits their usefulness[2].

Toxins may be thought of as naturally occurring drugs isolated from plants, bacteria or fungi. In general, they consist of two protein sub-units; an A and B chain. In the case of Ricin, isolated from the castor oil plant, the B chain allows the toxin to bind non-specifically to all cells as it contains a galactose binding site. This has to be either removed or modified so that this specificity is lost. Consequently, many of the toxin/antibody conjugates consist of the A chain of the toxin linked to the MoAb. It is the A chain of the molecule that interferes with protein synthesis within the cell, with one molecule thought to be cytotoxic once it enters the correct cytoplasmic compartment[3].

Linking the toxin to a MoAb is a major problem, as if the linkage is made too 'strong' the toxin will not be released from the MoAb when it enters the targeted cell which negates its toxic effect. If the link is too 'weak' it may fall off the MoAb before it binds to the tumour cells. One final problem with toxin/antibody conjugates is that the toxin element of the construct may be highly immunogenic in patients, once again limiting the potential of repeated therapy. This problem has been at least partially addressed by removing the carbohydrate moieties from the molecule.

Clinical use of targeted agents

Systemic administration

The human tumour mouse xenograft model was originally used to demonstrate the pre-clinical efficacy of targeted therapy. Animals with established tumours were administered isotope/antibody conjugates systemically and in many instances it was possible to eliminate tumours from the animals temporarily[4]. Using radiolabelled MoAbs, it is possible to demonstrate 6-10% of the injected dose of conjugate within the tumour with good tumour retention times[5]. In addition, specificity of uptake into the tumour is easily demonstrated by using 'specific' and 'non-specific' MoAbs radiolabelled with different isotopes.

Unfortunately, clinical studies in patients with a variety of malignancies have not held up the promise demonstrated by the animal models. The average uptake seen in solid tumour deposits in patients is 0.001% of the injected dose/gram[6]. Because of the low level of uptake, specificity is also more difficult to demonstrate, although it is clear that specific MoAbs usually accumulate to a greater degree in the tumour as compared to non-specific constructs.

At the level of uptake of radiolabelled MoAbs seen in solid tumours, the dose and dose rates calculated to be delivered are not of major therapeutic significance. Thus, targeting radionuclides by systemic administration compares poorly with conventional external beam radiotherapy[7]. Similar low levels of uptake into solid tumour deposits have been seen with toxins linked to MoAbs.

The reasons why antibodies enter human tumours in patients differently to those in animal models are still unclear. Obviously, there is a different volume of distribution in man as compared with mice. Non-specific entrapment of MoAbs by the reticuloendothelial system has been suggested to reduce the accessibility of antibody to tumour. Poor penetration of MoAbs from the systemic compartment to the interstitial space may also be important, as it must be remembered in the animal model the blood supply of the human tumour in the mouse is murine in origin. Human tumours have been calculated to have a higher interstitial pressure within them compared with normal organs, which limits diffusion of antibody into the tumour[8,9]. Finally, it has been postulated that a human antibody may be rapidly dehalogenated so that the isotope is not successfully targeted to tumour[10].

The poor penetration of MoAbs into solid tumour deposits has led to the exploration of smaller antibody fragments for therapy. Removal of the Fc region from the antibody reduces reticuloendothelial systemic uptake and results in a dimeric antigen binding fragment of approximately 100 kDa. This size reduction does not markedly enhance antibody uptake into tumour. Smaller Ig fragments result in the generation of monomeric rather than dimeric constructs. The binding affinities of these is therefore markedly less than whole antibody and $(Fab)_2$ fragments. Whilst increased levels of uptake of smaller fragments is observed in solid tumour deposits, it is still not sufficiently high to consider targeting as a viable approach to therapy. Whether CDRs will also show poor penetration into solid tumours remains to be determined, but these will almost certainly have lower affinities for tumour than naturally occurring MoAbs and their fragments. The level of tumour uptake that can be achieved with very small compounds can be estimated by reviewing data obtained with the radiopharmaceutical ^{131}I-*meta*-iodobenzylguanidine (*m*IBG). This is an analogue of adrenaline with a molecular weight of approximately 300 kDa. It is actively taken up into the cytoplasm of neuroblastoma cells[11] and into neurosecretory granules of phaeochromocytomas[12]. Uptake of this material into solid tumour deposits is only approximately one log above that seen with MoAbs[13].

Ways have been sought to increase uptake into solid tumour deposits. Injections into arteries feeding tumours has been shown to offer an advantage in uptake in animal models, but this again has not been seen clinically[14]. This naturally only offers a one pass advantage, but the concentration of antibody in the blood during this pass is higher than that achievable by venous administration.

The only reproducible therapeutic effects seen in patients given MoAb constructs systemically is when diffuse disease is targeted. Partial and complete remissions have been observed in this context, using either isotopes or toxins linked to MoAbs[15]. These have been noted in patients with neuroblastoma involving the bone marrow as well as in individuals with leukaemia/lymphoma[16-19]. Responses have been relatively short, however all of the studies have been undertaken in patients relapsing from conventional therapy.

The toxicity associated with systemically administered therapy differs, depending on whether isotopes or toxins are linked to the carrier molecules. MoAbs themselves have been given to patients in very high amounts and the only toxicity generally observed is due to the generation of an anti-mouse Ig response[20]. After several administrations of murine antibodies, patients can develop either mild, or occasionally severe, anaphylactic reactions. Usually, the anti-mouse Ig response noted is against Fc portion of the

molecule although anti-idiotypic responses have also been reported. These problems can be circumvented to some extent by the genetic engineering of murine MoAbs to humanize the molecules. This can be done by grafting murine heavy and light variable regions onto human constant regions. Alternatively, the molecules can be engineered so that only the antigen binding site of the MoAbs remains murine in origin, with the rest of the Ig molecule replaced by human counterparts. This dramatically changes the pharmacokinetics of the molecules which becomes very important if they are used as carrier molecules for either isotope or toxins. Humanized MoAbs remain in the circulation for a longer period of time than other murine equivalents. Unfortunately, the prolonged circulation times of humanized MoAbs does not markedly increase tumour uptake. Consequently, the therapeutic index between tumour and blood decreases, increasing toxicity without a concomitant increase in therapeutic efficacy.

The limiting toxicity associated with the administration of isotope/MoAb constructs is myelosuppression (^{131}I and ^{90}Y)[15,21]. In contrast, capillary leak syndrome limits the administration of toxin/antibody conjugates to patients[17]. Clearly, ways need to be found both to enhance the uptake of MoAb into tumour and reduce the toxicity of the targeted agents.

Compartmental administration of MoAb constructs

One approach to reduce toxicity and enhance antibody uptake to tumour has been to administer MoAbs to body compartments such as the peritoneum and cerebrospinal fluid (CSF). Targeting has been attempted to diffuse tumours such as malignant ascites in the peritoneum and metastatic medulloblastoma within the CSF. The pioneering work in this field was undertaken by Epenetos *et al.*[22] attempting to treat ascites arising from ovarian carcinoma with ^{131}I-MoAb (HMFG1). After interesting preliminary results, the group switched to ^{90}Y-MoAb constructs and have treated patients in the adjuvant setting. Excellent results from this study have resulted, although patient numbers are relatively small[23].

Within the CSF compartment we have attempted to treat a variety of malignancies presenting as diffuse leptomeningeal infiltrates. Here, antibodies have been given either by intraventricular or lumbar injection. The former route of administration is preferred, as there is a natural flow of CSF from the meninges to the sub-arachnoid space.

Patients were given a single injection of ^{131}I-MoAb and both toxicity and efficacy was determined[24]. The overall response rate in 41 patients teated with demonstrable leptomeningeal disease was 37%. Underlying this figure was considerable variability in response which depended upon the malignancy treated. No responses were noted in patients with gliomatosis. Of 15 patients (treated with ^{131}I-MoAb) with diffuse primitive neuroectodermal tumours (primarily medulloblastomas), the response rate was 33% with a median disease free interval of 11 months. In patients with CNS leukaemia six out of seven responses were noted, but these were only transitory, lasting 6-10 weeks[25].

Unfortunately, these studies have demonstrated that administration of MoAbs into either the peritoneal cavity or the CSF does not isolate them from the rest of the body. In both instances, the MoAbs enter the systemic circulation. Peak blood levels of 20-40% of the injected dose are found in the blood 36-54 h after MoAb administration (Figure 1). As a consequence, myelosuppression remains the limiting toxicity of this type of therapy. WHO grade 3/4 myelosuppression was observed in three out of nine patients receiving 60 mCi of ^{131}I-MoAb administered intrathecally[26]. As with other types of therapy, toxicity appears to be compounded by prior exposure of patients to high levels of cytotoxic drugs.

Despite the responses observed by targeting ^{131}I and ^{90}Y to diffuse tumours within body compartments, these isotypes are considered less than ideal for this purpose. ^{131}I has both a β and γ component. It is the β emissions that are important for targeted therapy. Ninety-five per cent of the energy of the β emissions (R_{95}) is dissipated 0.9 mm from the source. This means that for an average 15 μm cell, much of the energy will actually pass through the nucleus. The situation for ^{90}Y is considerably worse. This is a pure β emitter and has a R_{95} of 6.0 mm. Clearly, under these circumstances, maximum therapeutic efficacy can be brought about when targeting to clumps of cells where a cross-fire effect will be obtained. Isotope bound to one cell in a clump will exert its maximum

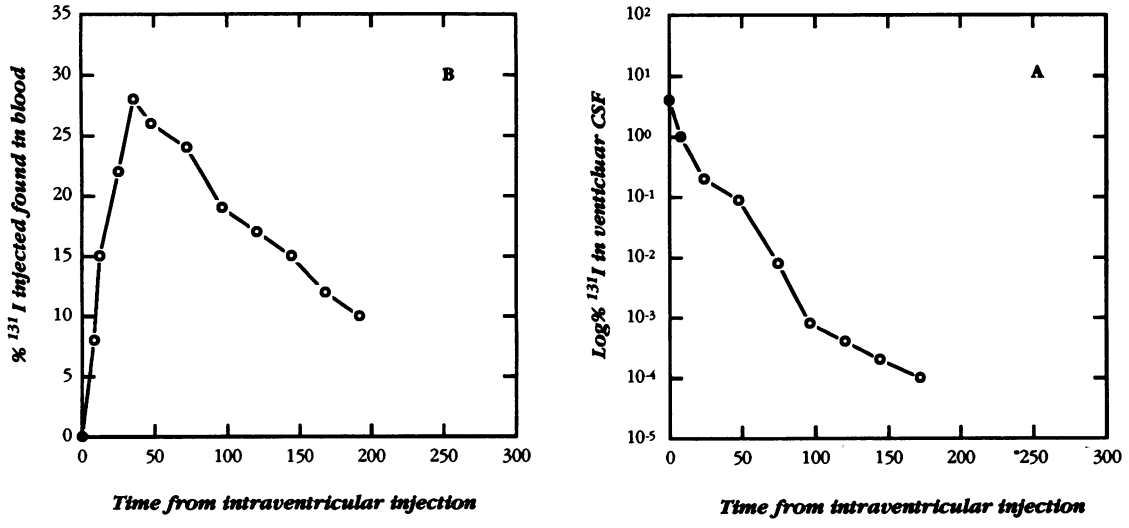
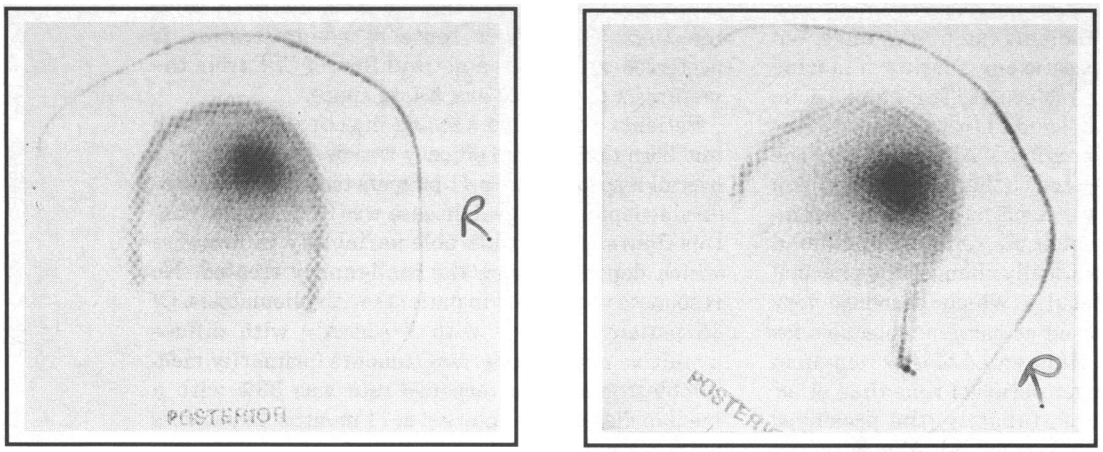


Figure 1. Typical distribution of ^{131}I-MoAb following intraventricular injection of ^{131}I-MoAb. (A) Clearance of ^{131}I from the ventricular CSF; (B) blood levels of ^{131}I following intraventricular injection of ^{131}I-MoAb



A B

Figure 2. Immunoscintigraphy of a patient receiving intratumoral injection of ^{131}I-MoAb. Image taken 11 days from administration of conjugate. (A) Anterior/posterior view; (B) lateral view

effect on another cell close by. These theoretical concepts have been well proven in vitro using tumour spheroid models. Thus, targeting becomes a compromise between delivering 'therapy' to small diffuse deposits so that antibody penetration does not become problematical and ensuring that a cross fire effect can occur by targeting to clumps of tumour cells.

There are alternative isotopes to those discussed above which are theoretically better to target to diffuse single cell disease. ^{67}Copper is a pure β emitter with low range emissions. ^{211}Astatine is an α emitter with even better characteristics for killing single cell disease. However, practical considerations such as chemical purity, isotopic purity and cost preclude these being clinically useful at the present time.

^{125}I has also been suggested to be a useful isotope for 'targeted' therapy. Within this context, the cytotoxic element of ^{125}I is the Auger emissions from the isotope. However, for these to be effective they have to be in very close proximity to the nucleus. Simply allowing ^{125}I to be targeted to membrane associated antigens outside the cell is, therefore, ineffective. The antibody needs to be internalized and, if possible, become associated with the nuclear membrane. Many of the MoAbs used to target leukaemic cells actually interact with the cytoplasm and in vitro studies have demonstrated that selective kill can be achieved with ^{125}I-MoAb constructs. Responses have also been noted in glioma patients given ^{125}I-MoAb binding to the epidermal growth factor receptor (EGFR). This is found in elevated levels in approximately 50% of gliomas studied. Some MoAbs are internalized when they bind to the EGFR and it is thought that they may also bind to the nuclear membrane where further receptors may be found[27,28].

Intratumoral administration of MoAbs

This is a highly specialized use of targeted therapy, clearly only applicable when tumours do not metastasize. One such disease is malignant glioma which is normally locally invasive rather than metastatic. The prognosis for patients with malignant glioma is poor. Conventional therapy consists of surgery and external beam radiotherapy and yet the mean survival of this group of individuals is around 11 months. Once relapse occurs, death usually results in 3-4 months. Further external beam radiotherapy is limited by toxicity to normal brain and, as a consequence, several studies have been undertaken using brachytherapy. Whilst increased responses have been noted in patients receiving implantation of radioactive seeds into their tumour, no large randomized studies of this approach to the treatment of glioma have been attempted. Clearly, the success of brachytherapy depends upon accurate positioning of the implants to give a uniform radiation dose to the tumour remaining after surgical debulking.

Over the last 2 years, we have attempted to replace conventional brachytherapy by targeted radiation therapy. Two groups of patients have been studied, those with a resection cavity remaining after surgical debulking and patients with a cystic element to their glioma. In our first study, ^{131}I-MoAb was instilled into the cavity/cyst via an Ommaya reservoir. A MoAb was chosen which recognizes the human neural cell adhesion molecule (NCAM), expressed on both normal brain and all gliomas tested. This allows the MoAb to bind to the wall of the resection cavity irrespective of whether this consists of normal tissue or tumour. By bringing the MoAb into close proximity to the wall, it irradiates a rim of tissue to a depth of the R_{95} of the antibody (for ^{131}I 0.9 mm). Furthermore, by choosing a MoAb that binds to normal tissue, its potential to diffuse should be limited by the high levels of antigen on the normal parenchyma surrounding the cavity (Figure 2). As diffusion occurs, then tumour cells present within the normal tissues close to the cavity should be irradiated by a cross-fire effect. However, it is not clear from our study of seven patients given ^{131}I-MoAb into a resection cavity/cyst how much diffusion actually occurs. We now plan to image patients using SPECT which gives a three dimensional reconstruction of the distribution of isotope in the cavity/tissues with respect to time.

In all patients studied, retention of the conjugate either within or close to the tumour cavity has been excellent. Patients have been given up to 60 mCi of ^{131}I-MoAb as a single injection without haematological toxicity. Peak blood levels of between 1 and 15% (mean 4.9%) of the injected dose have been observed in the blood 10-147 hours after administration of the conjugate (Figure 3; Papanastassiou

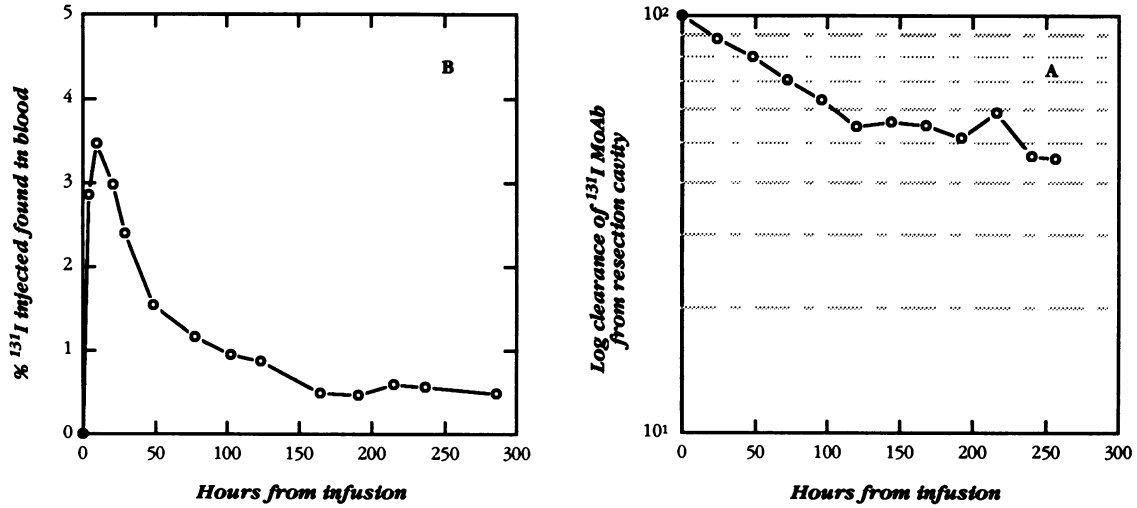


Figure 3. Typical distribution of ^{131}I-MoAb following intratumoral injection of ^{131}I-MoAb. (A) Clearance of ^{131}I from the resection cavity; (B) blood level of ^{131}I found after intratumoral injection of ^{131}I-MoAb

et al., unpublished). These are three to 15 times lower than those seen in patients receiving MoAb into the ventricular CSF. The primary toxicity associated with intratumoral therapy is raised intracranial pressure, seen clearly in two of the seven patients. One patient responded to steroids, whereas the other required a further debulking of tumour to resolve his symptoms. In this individual, the resected material was primarily necrotic. The only other toxicity seen in this patient group was a minor focal fit observed on administration of the conjugate in one individual and episodes of vomiting in another.

The number of patients entered into the study and the short-term follow-up makes assessment of the efficacy of therapy difficult. Both patients with cystic lesions demonstrated a marked reduction in the need to aspirate cyst fluid. For example, the first patient needed weekly aspirations prior to therapy, but following infusion of ^{131}I-MoAb, no further aspirations were needed for a period of 5 months. This patient died 9 months from therapy, while the second with a cystic lesion died 13 months after receiving the conjugate. Of the remaining five, two died 1-2 months from therapy, one with a tumour distant from the site of injection. The other three remained asymptomatic for a period of between 5 and 7 months. Two remain alive after further therapy.

As stated above, measurement of isotope levels within the body shows that the conjugate remains within or close to the site of injection. In addition to residence time, tumour doses depend on the degree of binding of the radioimmunoconjugate to either the cavity or cyst wall. Bearing in mind that the maximal β-particle range for ^{131}I is 0.9 mm and the dimensions of the cysts/cavities in these patients, any unbound isotope contributes little to tumour dose. Due to difficulties in directly measuring antibody binding, tumour doses have been calculated as a range, the lower and upper limits representing 0% and 100% binding respectively. These were high, ranging between 63 and 500 Gy (mean 226, median 128 Gy) for 0% binding and between 612 and 4630 Gy (mean 2125, median 1750 Gy) for 100% binding. These have been calculated assuming no parenchymal diffusion and represent, therefore, the mean dose delivered to a shell of cells approximately 1 mm thick around the tumour cavity/cyst.

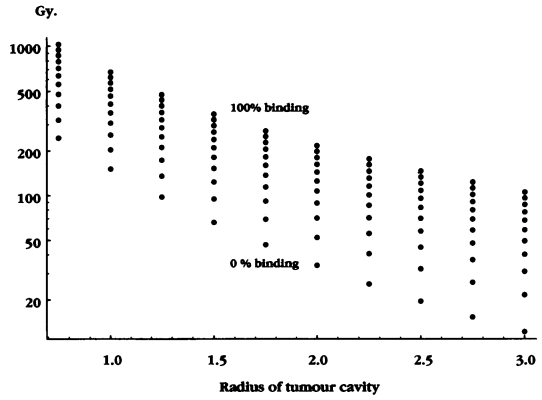


Figure 4. Calculated doses to glioma resection cavities after administration of ^{90}Y-MoAb. Dose delivered depends on both the size of the cavity and the degree of MoAb binding

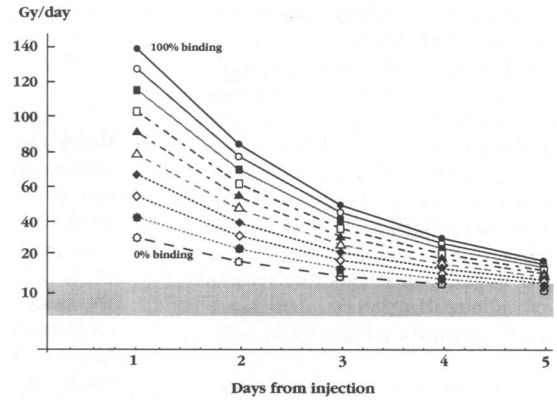


Figure 5. Dose rate achievable with targeted ^{90}Y-MoAb to resection cavities. Data is presented assuming a resection cavity of 2 cm radius. The shaded area represents dose rates obtained by solid source brachytherapy

Due to the limitations of ^{131}I, we have ended the pilot study and are currently injecting patients with the same antibody conjugated to ^{90}Y. In addition, the study design has been changed so that patients receive three injections of conjugate rather than one. Each patient receives 20 mCi of ^{90}Y-MoAb per injection and these are repeated at 4-6 week intervals (20 mCi of ^{90}Y being approximately equivalent in radiation dose to 60 mCi of ^{131}I). As with the patients

receiving ^{131}I-MoAb, primary toxicity in individuals receiving ^{90}Y conjugates is cerebral oedema, controllable with steroids. Dosimetric modelling again dictates that tumour dose is dependent upon the degree of MoAb binding and the volume of the cavity into which the conjugate is distributed. Figure 4 shows the calculated dose that can be achieved following instillation of ^{90}Y conjugates into tumour cavities.

Approximating the tumour cavity to a sphere of 2 cm radius, dose rates can also be calculated. Over the first 4 days of therapy, even assuming 0% binding, dose rates exceed those given by conventional brachytherapy (Figure 5). As the degree of binding increases, the tumoricidal dose given to a 6 mm rim of tumour and normal brain increases. It remains too early to comment on the efficacy of this therapy as this study only began 4 months ago. If phase I studies uphold the theoretical advantages of this approach to the treatment of gliomas, a randomized study incorporating targeted radiation into primary treatment of gliomas will be warranted. In one arm of the study patients will be treated by conventional surgery and external beam radiotherapy, whilst in the other arm this will be supplemented by a targeted component. However, before this is undertaken every effort should be made to maximize the binding of the conjugate to the tumour wall, as the efficacy of treatment is so dependent upon this parameter.

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